High Frequency Generalized Transduction by MiniMu Plasmid Phage

Boamin Wang,* Lin Liu,* Eduardo A. Groisman,† Malcolm J. Casadaban‡ and Claire M. Berg*

*Department of Molecular and Cell Biology, U-131, The University of Connecticut, Storrs, Connecticut 06268, and †Department of Molecular Genetics and Cell Biology, The University of Chicago, Chicago, Illinois 60637

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ABSTRACT

Deletion derivatives of phage Mu which replicate as multicopy plasmids, and also transpose and package like Mu, have been developed for the in vivo cloning of bacterial genes. We show here that these miniMu plasmid phage are also efficient at generalized transduction and that both in vivo cloning and generalized transduction of a given gene can be accomplished in a single experiment.

LYTIC growth of bacteriophage Mu entails repeated cycles of concomitant transposition and replication, resulting in single copies of Mu inserted at many sites in the host chromosome, often within only a few kb of each other. At the end of the lytic cycle, packaging is initiated near the Mu left (c) end and approximately 39 kb are incorporated into each phage head. The wild-type Mu genome is 37.5 kb, and thus about 1.5 kb of bacterial DNA adjacent to the Mu right (s) end is also normally packaged (TOUSSAINT and RESIBOIS 1983). Deletion derivatives of Mu, called miniMu, package correspondingly larger amounts of host DNA. Upon injection into a recipient cell, this host DNA can replace resident DNA in recA-mediated generalized transduction (Figure 1, III and IV). MiniMu-mediated generalized transduction is found at frequencies of $10^{-6}$ to $10^{-5}$ (FAELEN, TOUSSAINT and RESIBOIS 1979). Although miniMu-mediated generalized transduction is 10–100 times less frequent than P1-mediated generalized transduction, it is more useful for fine structure mapping and localized mutagenesis because it involves much smaller host DNA segments (20–30 kb instead of 90 kb).

There are two other ways in which miniMu can convey bacterial genes to recipient cells: (1) If the host DNA is bracketed by copies of miniMu in the same orientation, the entire miniMu-bacterial DNA-miniMu complex can be inserted by recA-independent transposition into the recipient genome, a process referred to as miniMu-duction (FAELEN, TOUSSAINT and RESIBOIS 1979). MiniMu-duction is useful for complementation studies because the transduced bacterial gene is added as a single copy by transposition; the recipient allele remains at the original site (CRONAN 1984). (2) In addition, if the miniMu element contains a plasmid replication origin, circularization by recombination between direct repeats of miniMu results in the formation of multicopy plasmids consisting of miniMu plus variable lengths of bacterial DNA (up to 25–30 kb) (GROISMAN and CASADABAN 1986; GROISMAN, CASTILHO and CASADABAN 1984).

We report here that miniMu elements which contain a plasmid origin of replication (like miniMu elements without an origin) are generalized transducing agents in Escherichia coli K12, and that generalized transduction is always more frequent than plasmid formation for a given gene. Insertional miniMu-elements were not recovered when a miniMu plasmid element was used. Our data illustrate that miniMu plasmid elements can be used for the in vivo cloning, and for the generalized transduction of a given gene in the same experiment.

MATERIALS AND METHODS

Chemicals and media: Specialized chemicals were purchased from Sigma Chemical Co., St. Louis, MO. Restriction endonucleases and the DNA size standards were purchased from Bethesda Research Laboratories, Gaithersburg, MD, and agarose was purchased from FMC Corporation, Rockland, ME.

Lennox (L) complex medium and Vogel and Bonner glucose-medium E salts were used as described previously (BERG and CURTISS 1967; BERG et al. 1979). The carbon source was glucose at 0.5%. Medium E was supplemented at the following levels where required: adenine (0.30 mM), L-arginine (0.10 mM), L-histidine (0.10 mM), L-isoleucine (0.15 mM), L-leucine (0.15 mM), L-methionine (0.067 mM), L-proline (0.26 mM), thiamine (0.006 mM), thymine (0.079 mM), L-tryptophan (0.10 mM) and L-valine (0.17 mM). Chloramphenicol (20 μg/ml), kanamycin (30 μg/ml) or streptomycin (200 μg/ml) was added where indicated.

Bacterial strains, plasmids and phage: Table 1 lists the E. coli K12 strains, phage and plasmids used. Cultures containing miniMu or Mucts were grown at 30°. Chloramphenicol (for Mud114042) or kanamycin (for Mud5005) was added to the growth medium to prevent the accumulation of plasmid-free segregants.

The two miniMu plasmid cloning vectors used have Mu genes A and B and the Mu ends needed for replication and transposition, but the central region of Mu containing morphogenetic and cell lysis genes is replaced in Mud14042 by the replication origin of P15A and the chloramphenicol resistance gene (cam) of Tn9 (GROISMAN, CASTILHO and
Class I: Packaging is initiated at the left (c) end of Mu. A complete Mu genome is packaged. Infection results in lytic growth or lysogenization.

Class II: Only bacterial DNA is packaged. Packaging is probably initiated at sites which resemble Mu packaging initiation sites. The only productive fate is generalized transduction, with the donor chromosomal fragment replacing the recipient allele.

Classes III and IV: Packaging is initiated at the left (c) end of miniMu. One complete miniMu genome plus DNA contiguous with the right (s) end of miniMu is packaged. If the right portion is entirely bacterial DNA (III) or bacterial DNA plus part of Mu or miniMu in the right to left (s to c) orientation (IV), the only productive fate is generalized transduction with the donor chromosomal fragment replacing the recipient allele (associated with loss of all Mu DNA).

Class V: Packaging is initiated at the left (c) end of miniMu. One complete miniMu genome plus bacterial DNA and at least a portion of Mu or miniMu in the left to right (c to s) orientation is packaged. Infection results in introduction of a hybrid DNA segment which has a complete miniMu genome at one end and a similarly oriented Mu or miniMu segment at the other end. Consequently, homologous segments can pair, crossover, and circularize to form a plasmid. Subsequent recombination with the chromosome and loss of the unstable plasmid can yield a generalized transductant, indistinguishable from those produced by class II, III or IV events.

Casadaban (1984), and in Mud5005 by the replication origin of ColEl and the kanamycin resistance gene (kan) of Tn5 (Grossman and Casadaban 1986). The Mudl14042 genome is 16.7 kb and the Mud5005 genome is 7.9 kb. Consequently, 22 kb or 31 kb, respectively, of DNA that had been adjacent to the right (s) end of the parental miniMu insertion is also packaged. That adjacent DNA may be entirely bacterial in origin (Figure 1, III) or may extend into a second nearby Mu or miniMu element (Figure 1, IV or V).

Phage manipulations: To prepare lysates, overnight cultures grown in L broth plus chloramphenicol or kanamycin (to about 10^5/ml) were diluted 10^5-fold into fresh L broth plus 10 mM MgSO_4_and incubated with aeration at 30^o until the cells were growing exponentially and had reached a titer of about 10^9 cells/ml. At that time they were shifted to 42^o for 20 min to induce lytic growth and then to 37^o for 1–2 hr until clearing or foaming (depending on the strain) was observed. In later experiments, the antibiotic was not removed from the medium until the culture was shifted to 37^o, a procedure which seems to give higher titers of antibiotic resistant plasmids.

Mucts lysogens were constructed by spotting a fresh Mucts lysate on a bacterial lawn on L agar and incubating at 30^o overnight. Cells were streaked from the center of plaques onto the same medium and the resultant colonies were tested for temperature sensitivity at 42^o. Mucts-miniMu double lysogens were constructed by infecting a Mucts lyso- gen with a fresh Mucts-miniMu lysate and selecting for chloramphenicol or kanamycin resistant transductants at 30^o.

MiniMu transduction and transductant testing: Freshly prepared lysates were used to infect Mucts lysogenic derivatives of CBK812, CBK741 or χ697 at multiplicities of infection of 0.2–2.0 plaque forming units (pfu) per cell. After allowing 1–2 hr for phenotypic expression at 30^o, the cells were plated on appropriately supplemented minimal medium with or without kanamycin (Mud5005) or chloramphenicol (Mudl14042). Transductants were characterized without purification to avoid plasmid loss in the absence of antibiotic selection.
Generalized Transduction by MiniMu

**TABLE 1**

**E. coli K12 strains and Mu derivatives used**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Derivation or source</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Bacteria</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CBK741</td>
<td>$\Delta$(proB-lac) thyA ilvE12 avaA23::Tn5</td>
<td>WHALEN, WANG and BERG (1985)</td>
</tr>
<tr>
<td>CBK801</td>
<td>XPh43 ilvE720::Tn5</td>
<td>Transduction by P1</td>
</tr>
<tr>
<td>CBK812</td>
<td>XPh43 ilvE12</td>
<td>Transduction by P1</td>
</tr>
<tr>
<td>MH2923</td>
<td>$\Delta$</td>
<td>CSONKA et al. (1981)</td>
</tr>
<tr>
<td>$\chi$697</td>
<td>ara-14 leuB6 azi-6 tonA23 lacY1 $\Delta$sx-67 purE22</td>
<td>BERG and CURTISS (1967)</td>
</tr>
<tr>
<td></td>
<td>supE44 galK2 trpE38 his-208 argG77 rpsL109 xyl-5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>mlc-2 ilvA681 metA160 thi-1</td>
<td></td>
</tr>
<tr>
<td>XPh43</td>
<td>$\Delta$(argF lacPOZYA)U169 trp $\Delta$(brnQ pheA proC pheB pheR)</td>
<td>BRICKMAN and BECKWITH (1975)</td>
</tr>
<tr>
<td>B. Phage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mucts</td>
<td>$\Delta$</td>
<td></td>
</tr>
<tr>
<td>Mud114042</td>
<td>$\Delta$</td>
<td></td>
</tr>
<tr>
<td>Mud5005</td>
<td>$\Delta$</td>
<td></td>
</tr>
<tr>
<td>C. Plasmids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pEG109</td>
<td>$\Delta$(lacZ)</td>
<td></td>
</tr>
<tr>
<td>pEG5005</td>
<td>$\Delta$(lacZ)</td>
<td></td>
</tr>
<tr>
<td>plF003, plF016</td>
<td>$\Delta$(lacZ)</td>
<td></td>
</tr>
<tr>
<td>pF065, pF066,</td>
<td>$\Delta$(lacZ)</td>
<td></td>
</tr>
<tr>
<td>plF067</td>
<td>$\Delta$(lacZ)</td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 2**

Phage, plasmid and generalized transductant formation from a Mu-miniMu plasmid lysogen

<table>
<thead>
<tr>
<th>Expt. No.</th>
<th>Recipient</th>
<th>Selected marker</th>
<th>Pfu/ml ($\times 10^9$)</th>
<th>Total ($\times 10^9$)</th>
<th>$\Delta$us* or Arg* ($\times 10^9$)</th>
<th>Kan$^R$ generalized transductants ($\times 10^9$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CBK812Mucts</td>
<td>$\Delta$</td>
<td>0.88</td>
<td>0.51</td>
<td>0.96</td>
<td>2.3</td>
</tr>
<tr>
<td>2</td>
<td>CBK812Mucts</td>
<td>$\Delta$</td>
<td>0.77</td>
<td>0.44</td>
<td>0.77</td>
<td>1.9</td>
</tr>
<tr>
<td>3</td>
<td>$\chi$697Mucts</td>
<td>$\Delta$</td>
<td>1.8</td>
<td>2.3</td>
<td>0.72</td>
<td>2.7</td>
</tr>
<tr>
<td>4</td>
<td>$\chi$697Mucts</td>
<td>$\Delta$</td>
<td>2.8</td>
<td>1.1</td>
<td>0.55</td>
<td>2.7</td>
</tr>
</tbody>
</table>

* Phage was prepared on MH2923/pEG5005 Mucts.

Analysis of plasmid DNA: DNA was prepared by the alkaline lysis method of Birnboim and Doly as described in MANIATIS, FRITSCH and SAMBROOK (1982).

Enzyme assays: Transaminase C was assayed as described previously (WHALEN and BERG 1982): specific activity is expressed as nanomoles of pyruvate produced per minute per milligram of protein. Chloramphenicol acetyltransferase was assayed as described by SHAW (1975): specific activity is expressed as micromoles of chloramphenicol acetylated per minute per milligram of protein.

RESULTS

Efficiencies of transduction: MiniMu-Mu helper lysogens were thermally induced and the frequencies of miniMu plasmids and of the two classes of transductants were determined: the titer of plaque forming units (pfu) was generally about $10^9$/ml and that of antibiotic resistant plasmid transductants was about $10^{-4}$/pfu ($10^9$/ml) for Mud5005 (Kan) (Table 2) and $10^{-3}$/pfu ($10^6$/ml) for Mud114042 (Cam) (data not shown). Transductants for specific genes were found at frequencies greater than $10^2$/ml for plasmid transductants (Figure 1, V) and $10^3$/ml for generalized transductants (Figure 1, III and IV) (Table 2).

The relative frequencies of plasmid and generalized transductants for a number of genes were determined using a multiauxotrophic recipient. All seven genes tested were transducible, but at very different frequencies (Table 3). The number of generalized transductants exceeded the number of plasmid transductants for each of the markers tested, with the ratios of generalized to plasmid transductants ranging from 1.7 to 37. The relative frequencies of the two transductant classes varied from lysate to lysate and from gene to gene, in part because miniMu plasmids are unstable and small variations in the growth conditions can drastically affect their number in the host cell and...
hence the relative recoveries of generalized transductants (Figure 1, II–IV) and of plasmids (Figure 1, V). The gene to gene differences may, in addition, reflect nonrandom Mu insertion.

**MiniMu plasmid loss and marker exchange:** MiniMu plasmids are unstable and must be maintained under selective conditions (Groisman, Castilho and Casadaban 1984). We found, for example, that if transductants selected in the absence of antibiotic were purified by restreaking once on the same medium, greater than 95% of the colonies were plasmid-free generalized transductants, while if the colonies were tested without purification, a significant frequency of plasmid transductants was found (Tables 2 and 3).

Replacement of the chromosomal allele by the plasmid-borne allele (marker exchange) requires a pair of crossovers in the homologous segments bracketing the mutant allele. The effect of size of the region of homology was tested in CBK741 with miniMu-avtA+ plasmids carrying chromosomal fragments of different sizes. After about 12 generations of growth in minimal medium lacking valine and chloramphenicol (which selects for avtA+, but permits loss of the plasmid) CBK741/pIF003 (in which avtA+ is bracketed by about 2.8 and 0.7 kb) yielded 36 plasmid-less avtA+ colonies out of 100 tested, while CBK741/pIFO16 (1.4 and 0.6 kb) and CBK741/pIF065 (5.7 and 0.1 kb) (M. Wang, L. Liu and C. Berg, unpublished results) yielded none. After about 18 more generations of growth in the same medium, CBK741/pIFO16 yielded one plasmid-less avtA+ colony out of 100 tested, but CBK741/pIF065 again yielded none. These data indicate that the ease of replacing the chromosomal allele by the plasmid-borne allele depends upon the lengths of the flanking regions of homology. Since the chromosomal avtA mutation is due to a Tn5 insertion, pairing may be impeded, and marker replacement involving a chromosomal point mutation might be more efficient.

**Are miniMu plasmid elements capable of miniMu-duction?** In the experiments presented in Tables 2 and 3, antibiotic-sensitive transductants were considered to be due to generalized transduction, while antibiotic-resistant transductants were considered to be due to the formation of miniMu plasmids (Figure 1). Although plasmids can be routinely recovered from antibiotic-resistant transductants (Groisman and Casadaban, 1986; Groisman, Castilho and Casadaban 1984) (M.-D. Wang, L. Liu and C. M. Berg, unpublished results), and plasmids with a high copy number origin of replication are not normally found integrated into the chromosome (Kingsbury and Helinski 1973), the presence of occasional miniMu-ductants, in which a miniMu-host-miniMu complex
transposes as a unit into the chromosome, had not been excluded.

To screen for possible miniMu-ductants we used the \textit{avtA} gene (which encodes the alanine-valine transaminase) because the phenotype it confers is copy-number dependent: \textit{ilvE- avtA-} strains require isoleucine and valine, while \textit{ilvE-} strains with a single copy of \textit{avtA+} require only isoleucine (Whalen and Berg 1982), and \textit{ilvE-} strains with \textit{avtA+} on a multi-copy plasmid do not require either amino acid (M.-D. Wang and C. M. Berg, unpublished data). This difference (due to isoleucine synthesis at physiologically significant rates when this transaminase is overproduced) permitted a rapid screen of valine-independent (\textit{avtA+}) transductants for any which might have been due to insertion of the Mu-\textit{avtA+} complex into the chromosome. Only three out of 2050 valine-independent chloramphenicol-resistant \textit{Mud114042} transductants of CBK741 (\textit{ilvE- avtA-}) were found to require isoleucine (Table 4), indicating a low level of \textit{avtA} expression. Their transaminase activities ranged from 10 to 40% of that found in the other chloramphenicol-resistant \textit{avtA+} transductants, but was from two to eight times that found in haploid \textit{avtA+} strains (Table 5).

MiniMu copy number in these three transductants was assessed by measuring the level of chloramphenicol acetyltransferase activity encoded by the miniMu \textit{cam} gene: two transductants had the high levels characteristic of \textit{Mud114042} plasmids, while the third had about one-tenth as much, consistent with the reduction observed in transaminase activity (Table 5). Plasmid DNA was readily obtained from the two strains with high chloramphenicol acetyltransferase activity, but only with difficulty from the third. This indicated that all three strains contained \textit{Mud114042-avtA+} plasmids, but that the plasmid was reduced in copy number in one of them (pIF067). When the plasmid DNA from the aberrant strains was analyzed, pIF065 and pIF066 were found to have one miniMu-chromosomal DNA juncture very close to an end of \textit{avtA}, while pIF067 did not (data not shown). The low \textit{avtA+} expression in CBK741/pIF067 is probably due to the decreased plasmid copy number, while the low expression in CBK741/pIF065 and CBK741/pIF066 is probably due to a partial defect in \textit{avtA}. These data suggest that the proximity of the juncture to \textit{avtA} in pIF065 and pIF066 is responsible for the isoleucine requirement of strains carrying these two plasmids. The absence of miniMu-duction (0/2050 \textit{avtA+ cam+} transductants tested) indicates that under our conditions miniMu elements which contain a plasmid origin of replication form viable insertions into the chromosome at less than $5 \times 10^{-4}$ the frequency at which they form plasmids.

### DISCUSSION

We show here that the miniMu plasmids developed for \textit{in vivo} shotgun cloning (Groisman and Casadaban 1986; Groisman, Castilho and Casadaban 1984) are also useful for generalized transduction (Tables 2 and 3). While generalized transduction by P1 is found at frequencies of $10^{-3}$ to $10^{-4}$ (Caro and Berg 1971), generalized transduction by a mini-Mu helper lysate is about 10-fold lower (Table 1) even though each miniMu phage carries bacterial DNA. Nonetheless, usable numbers of transductants can be obtained even without concentrating the transduced cells before plating (Table 3). The relatively low transductant frequency, despite the many virions containing bacterial DNA in a miniMu-Mu lysate, probably reflects inefficient recombination into the chromosome by the small bacterial fragments packaged with miniMu elements.

MiniMu elements that lack a plasmid origin of replication have not been used extensively for generalized transduction, in part because the analysis of transductants is complicated by the occasional insertion of a complex transposable element (miniMu-bacterial gene-miniMu) (miniMu-duction) (Cronan 1984; Fahlen, Toussaint and Resibois 1979). Although no miniMu-ductants were found in these experiments, it
is probable that miniMu plasmid elements could mediate miniMu-duction under conditions in which the plasmid origin of replication is repressed. We have found, for example, that Mud5005 will function as a transposon in a strain already carrying a multicopy plasmid of the same incompatibility group (E. Groisman and M. Casadaban, unpublished results).

Since transducing particles from a Mu-miniMu double lysogen that contain a headful (39 kb) of bacterial DNA represent only a small fraction of all transducing particles (introduction), the maximum size of bacterial DNA in most transducing particles is 22 kb for MudI4042 and 31 kb for Mud5005 (Figure 1, III). Both larger and smaller Mud phages have been constructed (Groisman and Casadaban 1986) (L. Liu, N. Vartak and C. M. Berg, unpublished results). Therefore, the maximum size of transducing fragments in a lysate can be predetermined by choice of the appropriate miniMu plasmid element. This is useful for localized mutagenesis. For example, generalized transduction by miniMu transposons (Cronan 1983) and by miniMu plasmids (B. Wang, L. Liu and C. Berg, unpublished results) has been used successfully to isolate Tn10 insertions very tightly linked to genes of interest.

We show here that miniMu plasmids containing a plasmid origin of replication are useful for generalized transduction and that transductants can arise in a primary event following introduction of a segment of bacterial DNA (Tables 2 and 3) or, secondarily from an established, but unstable, plasmid by marker exchange and loss of the plasmid. The use of miniMu with a multicopy plasmid origin ensures that all chromosomal transductants are haploid, since miniMu-ductants are not recovered (Table 4). Thus, miniMu plasmid vectors should be more useful than other miniMu elements for generalized transduction, especially of tightly linked markers and for localized mutagenesis.

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LITERATURE CITED


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